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LIFE SPAN AND LYMPHOMA-INCIDENCE OF MICE INJECTED AT BIRTH WITH SPLEEN CELLS ACROSS A WEAK HISTOCOMPATIBILITY LOCUS

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The present study concerns one of several experimental "models" devised as partial tests of the immunologic theory of aging. This theory postulates that immunogenetic diversification of the body's cells takes place with time, that such diversification leads to prolonged minor grade histo-incompatibility reactions, and that these reactions result in or contribute to aging.[1,2] The emphasis here is on minor grade or weak reactions. For example, skin allografts exchanged between mice of the congenic strain pairs C_3H (H-1^a) and $C_3H.K$ (H-1^b) show mean survival times of about 30 days (H-1^b skin → H-1^a recipients) and 90 days (H-1^a skin → H-1^b recipients) respectively.[3] In comparison to many other histocompatibility gene combinations recently evaluated in mice, these H-1 alleles represent a "weak" and a "very weak" difference, depending on the direction of the immune reaction. Now injection of newborn mice with adult spleen cells across such a congenic strain combination involving weak transplantation antigen (for example adult H-1^b spleen cells → H-1^a newborns) might lead to a prolonged but subclinical histo-incompatibility or graft-versus-host reaction. If this indeed occurred, accelerated aging should result, according to the theory. It is this "model" experiment with which we are here concerned, and in the adult graft-versus-neonatal-host experiments it will be noted that the test is in the direction of "very weak" incompatibility. The life

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span and disease patterns of such test mice (H-1^b spleen cells → H-1^a neonates) as well as control mice (H-1^a spleen cells → H-1^a neonates) were determined.

Material and Methods

Two congenic strains of C_3H mice identical at all histocompatibility loci except H-1 in the first linkage group were employed. These were C_3H (H-1^a) and $C_3H.K$ (H-1^b), obtained from Dr. George Snell at the Jackson Memorial Laboratory, Bar Harbor, Maine. For purposes of this paper they will be designated according to their H-1 alleles as H-1^a and H-1^b mice. Forty-seven neonatal H-1^a mice born over a 3- to 4-day period were given injections by the intracardiac route according to the method of Grazer [4] of about 2.5×10^6 spleen cells obtained from 3-month-old H-1^b male mice. Fifty-seven newborn H-1^a mice were similarly given injections of adult H-1^a male cells. All injections were made within 24 hours and generally within 12 hours of birth.

The populations were weighed at weekly intervals for 3 months, thence at monthly intervals for another 5 months. At the time of weaning the animals were divided according to sex and retained in a density of 6 animals per cage. The populations were kept in the same room and in adjacent positions on the cage rack. They were fed an ordinary commercial mouse chow. All animals were allowed to live out their natural life spans. At death, total body weights and spleen weights were determined and all animals received a gross necropsy examination. Except in cases of advanced autolysis, microscopic examination of liver, spleen, usually kidney, and other organs as seemed appropriate was accomplished.

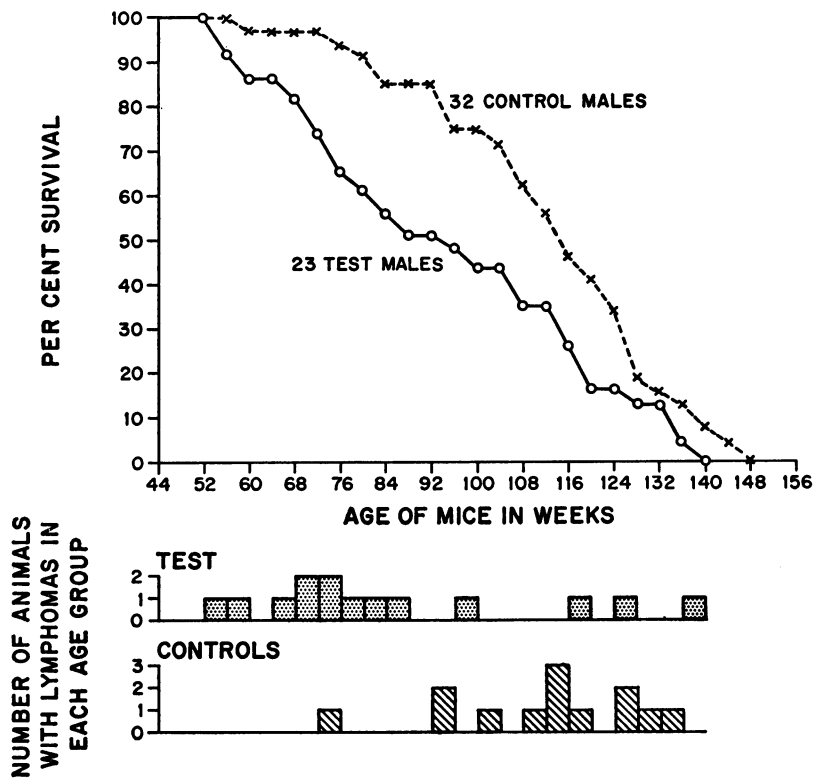
Results

There was no evidence of runt disease in the test population as judged by external examination or weight measurements during the 8 months after birth. In fact both populations appeared indistinguishable until about 1 year of age. At this point the male test mice began dying at an accelerated rate as compared to the controls (Text-fig. 1). This accelerated death rate was associated with a high incidence of lymphomatous disease (14 of 23 mice), as shown in Text-figure 1. Thirteen of the 32 male control mice also died of lymphomatous disease, but at a much more advanced age (Text-fig. 1). Excluding lymphoma, the mean survival of 9 test male mice was 106 weeks; of 19 controls, 122 weeks.

Lymphoma when present was always manifest by a greatly enlarged spleen (Fig. 1). Lymphadenopathy attributable to lymphoma was seen in 4 test and 2 control mice, thymic enlargement in 1 test and 4 control mice (Figs. 1 and 2). Microscopic examination always revealed extensive lymphoid infiltrates in the liver and spleen, with occasional involvement of other organs (Figs. 3 and 4). The histologic pattern resembled that designated as lymphocytic neoplasm by Dunn.[5] Deaths not associated with lymphoma were due to multiple causes, chiefly respiratory infection, but including one neurogenic sarcoma and one squamous cell carcinoma. Six control mice and one test mouse died of

hepatoma. In a number of mice no clear-cut cause of death could be established from necropsy examination.

Female H-1[a] mice characteristically show a very high incidence of spontaneous mammary carcinoma and die at 9 to 15 months of age. There was no difference in the present study between the survival curves



TEXT-FIG. 1. Life table and age-related distribution of lymphomas in test (H-1[b] → H-1[a]) and control (H-1[a] → H-1[a]) populations of mice injected at birth with adult splenic cells.

of test and control females. The majority in both categories succumbed to mammary carcinoma at a comparatively early age, and they were thus not suitable for estimates of rates of aging by life table statistics. Of the female mice, 4 of the 24 test animals died of lymphoma and 15 of mammary carcinoma; of the 25 control mice none developed lymphoma and 18 died of carcinoma. Probably many more of the females would have developed lymphoma had they not succumbed to the earlier appearing tumor.

DISCUSSION

It should be emphasized that in C_3H mice in general, and including

both of the present strains, reticuloendothelial neoplasm is quite uncommon. C_3H strains are indeed often used in studying virus-induced leukemias precisely because they do have such a low spontaneous incidence of this disease. This negligible incidence of leukemia and lymphoma obtains also with non-injected C_3H (H-1^a) mice kept into advanced age in our own populations. The finding of a very high lymphoma incidence in both test and control populations, albeit at quite different average ages, was therefore unexpected. Our experiment in its present form is susceptible to a number of different interpretations. These can be individually stated as follows:

First Possibility. The lymphomas in the test mice were a manifestation of transplantation disease mechanisms in that the injected H-1^b cells assumed neoplastic characteristics after long residence in the tolerant H-1^a host. Lymphoma was the final result of a prolonged graft-versus-host reaction across very weak loci. Sjögren, Hellström and Klein,[6] and Habel[7] have presented evidence that malignant neoplasm cells may contain histocompatibility antigens different from those of the host animal. In work not yet fully reported Schwartz[8] noted the occurrence of lymphomas in F_1 hybrid mouse recipients given injections of parental spleen cells and observed for an 8- to 15-month period. He interprets these lymphomas as arising consequent to disturbed immunologic mechanisms.

The high incidence of lymphoma in our control mice (albeit at a much later age than in the test mice) appears anomalous in terms of an immunologic interpretation, but might reflect the existence of residual heterozygosity of the injected adult H-1^a cells. Since we are specifically concerned here with very weak histocompatibility differences, this viewpoint is not untenable. It is also conceivable that the lymphomas in control mouse recipients resulted from an altered reticuloendothelial homeostasis due to "homing" of large numbers of injected adult cells on neonatal lymphoid centers. Leduc and Wilson[9] produced a small number of hepatomas in mice by transplanting normal donor liver tissue into the spleens of recipients—all mice being of the same inbred strain. The hepatomas appeared not in the spleens but in the draining livers. They were transplantable and were considered as true neoplasms. These investigators' experiment could be viewed as involving weak loci and residual heterozygosity, similar to the situation postulated for our control mice. The primarily immunologic interpretation of our total experiment given above would be consistent with Tyler's immune theory of cancer.[10]

Second Possibility. The injected material contained a lymphoma-inducing virus. While at first sight this might seem unlikely in view of

the exceedingly low incidence of spontaneous leukemia in C3H mice, Rowe [11] states that a mouse strain may harbor a leukemia virus without itself showing a high incidence of leukemia. It is well established that leukemia and polyoma viruses are ordinarily capable of inducing tumorigenesis only following injection into neonates. Many "normal" adults have antibody to polyoma virus, but very rarely develop tumors.[11] Rivière and associates [12] produced lymphomas in 15.5 per cent of strain XLII mice by injecting recipients at birth and at weekly intervals thereafter with DNA extracted from normal C3H mice. The peak lymphoma incidence was at 22 months of age. Their experiment might be regarded as a transformation reaction [13] as well as induction of virus multiplication.

In any case, a viral interpretation does not satisfactorily explain the great difference in age-specific incidences of lymphomas in our test and control populations. With two congenic mouse strains it seems unlikely that two separate viruses producing identical morphologic disease patterns would occur—both viruses failing to produce disease in the normal adult population, yet showing markedly different age-incidences of induced disease following injection into neonates. If a viral etiology is involved, one would assume the same virus to be responsible in both test and control populations. The more rapid development of lymphoma in the test mice therefore would suggest the action of some additional and non-viral factor.

It might be that minimal or subclinical transplantation disease was produced in the test newborn mice, allowing the appearance of virus-induced lymphoma at a younger age than in the control mice. In general, runt disease or transplantation disease involves thymic injury,[14] and thymic injury usually protects against virus-induced leukemia.[11,15] In some instances, however, including at least one strain of C3H mice, thymectomy or thymic injury may not influence leukemia incidence,[16] and in C57BL mice the thymus actually plays a role in resistance to polyoma virus oncogenesis.[17] An explanation involving virus plus subclinical transplantation disease is therefore not inconsistent with known facts, although it involves a number of unproved assumptions.

A final possibility linked with virus oncogenesis is that we are dealing with a virus whose lymphoma-inducing effects are more or less specific for the physiologic age of the mouse. The injected cells in the test mice transmitted a virus, but also led to accelerated aging in accordance with what was expected of an experimental "model" for the immunologic theory of aging. The effect of accelerated physiologic aging in the test mice would be to shift the peak incidence of lymphoma to a chronologically earlier period. It is interesting that the curves in Text-figure

1 recall the life-shortening effects caused by low-dosage irradiation. Furthermore, with such irradiation a shift in tumor incidence to a younger age and an absolute increase in leukemia is characteristically observed. The age-related incidence of cancer is indeed considered one of the best estimates of "physiologic" aging.[18]

There is no precise way of deciding between the various above listed possibilities on the basis of data at hand. Our view is that transplantation mechanisms are certainly involved, and that, in addition, a viral etiology may or may not be involved. Further experiments utilizing many different genetic combinations of mice are in progress and will be reported at a later date. It can, however, be said at this time that the present experiment, as detailed, appears quite repeatable. It does not represent a temporary viremia or other chance event. H-1^a mice given injections at birth 15 months ago with H-1^b cells are again showing a high incidence of lymphomatous deaths commencing at about 1 year of age.

Summary

Newborn congenic C$_3$H mice receiving injections across a weak (H-1$^b \rightarrow$ H-1^a) histocompatibility barrier exhibited life-shortening and a high incidence of lymphomatous disease during mid-adult life. In control experiments (H-1$^a \rightarrow$ H-1^a) lymphomatous disease developed much later in life. Both mouse strains when not given injections as newborns manifest only a negligible incidence of lymphoma or leukemia. These findings are variably interpretable in terms of oncogenic virology, and of the immunologic theories of aging and cancer by analogy with transplantation disease mechanisms.

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[*Illustrations follow*]

Legends for Figures

Fig. 1. Massively enlarged spleen characteristic of the mouse lymphoma observed in this study. Enlarged lymphomatous mesenteric lymph nodes are also shown.

Fig. 2. Marked cervical lymphadenopathy due to lymphoma is seen in a small number (4 test and 2 control) of mice.

Fig. 3. Extensive lymphoid infiltration appears in the liver of a test mouse dying at 66 weeks of age. Hematoxylin and eosin stain. × 90.

Fig. 4. Lymphoma of the spleen in a test mouse dying at 58 weeks of age. Hematoxylin and eosin stain. × 310.